20.109 MOD1
Measuring Genomic Instability

Fall 2022
Day 3

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Professor of Biological Engineering

Previous lecture -

Cancer is caused by acquired traits; mutations make new traits possible

Overview of the steps of BER

Chemistry of nucleotide addition (on the blackboard & in handout)

Story of water contamination and arsenic

How PARP helps BER

A careful look at the major steps of BER

γH2AX as a Marker of DNA Damage

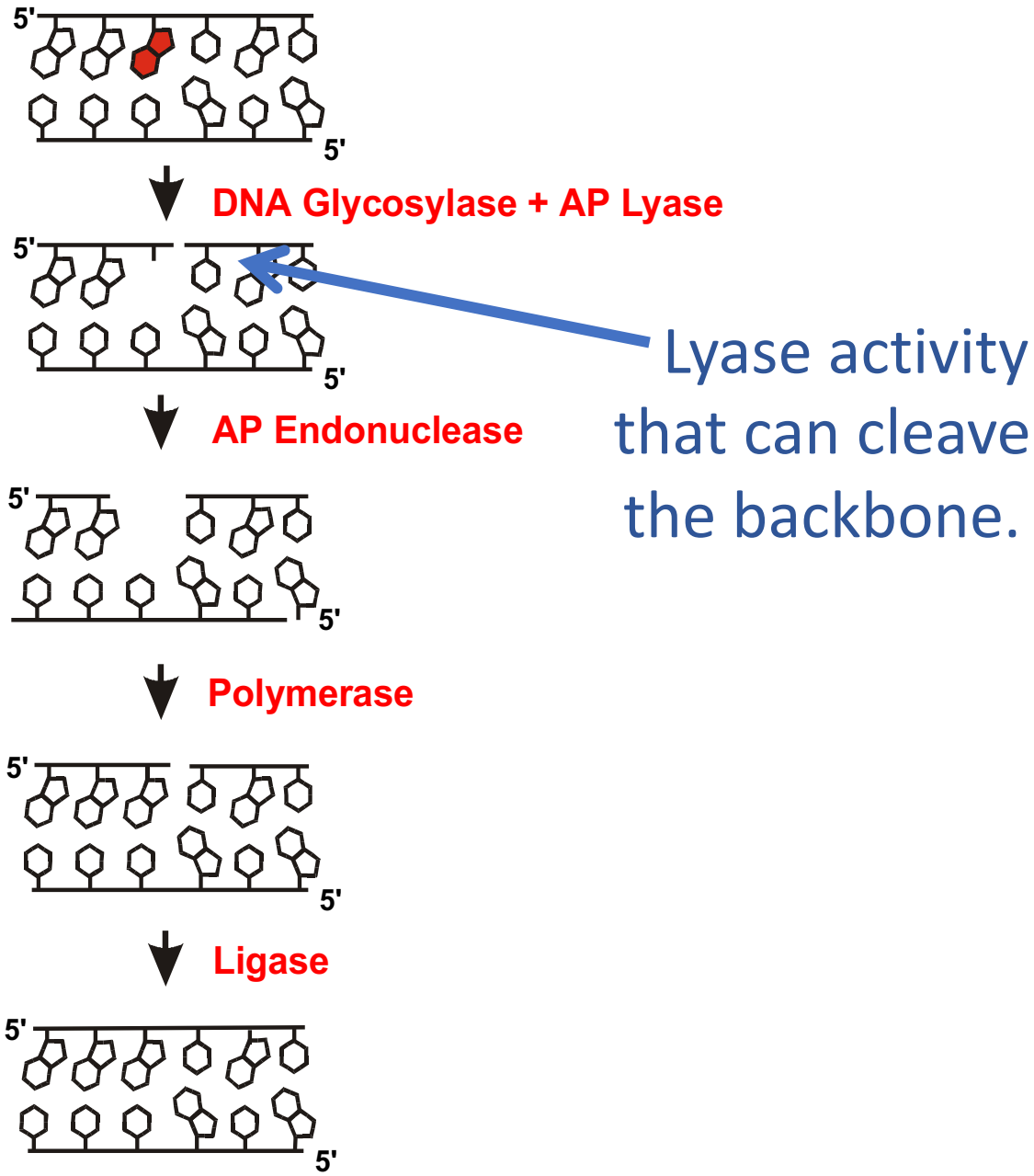
Interlude

Base Excision Repair (BER)

Base Excision Repair

8-oxoguanine DNA Glycosylase (OGG1)

Removes the damaged base by cleaving the glycosylic bond.

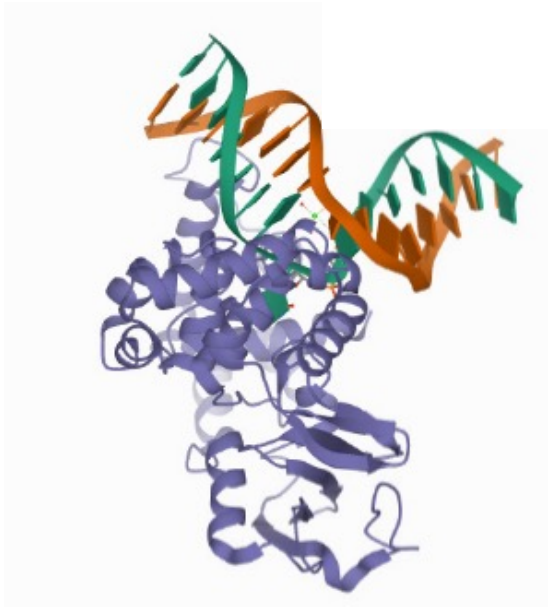


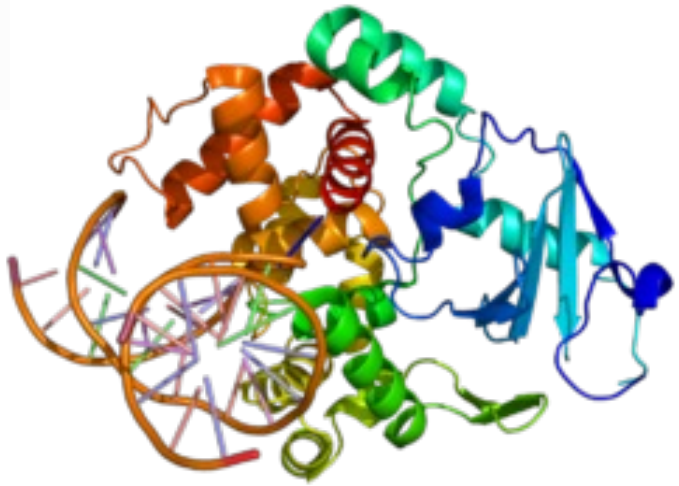
8-oxoguanine DNA Glycosylase (Ogg1)

Removes the damaged base.

Cleaves the backbone

Leaves behind an abasic site with a a nick.





Mutations in OGG1 are Associated with Increased Risk of Breast Cancer

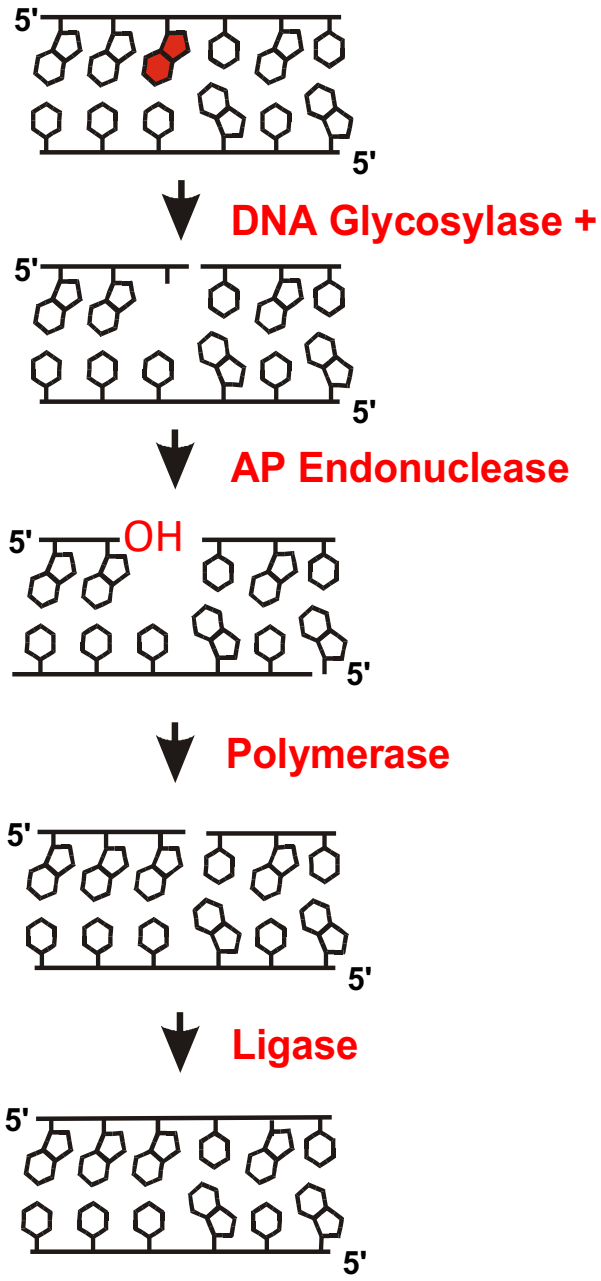
In some cases, the risk is > 15X Higher

Base Excision Repair

AP Endonuclease

"Cleans the end" (removes the abasic sugar)

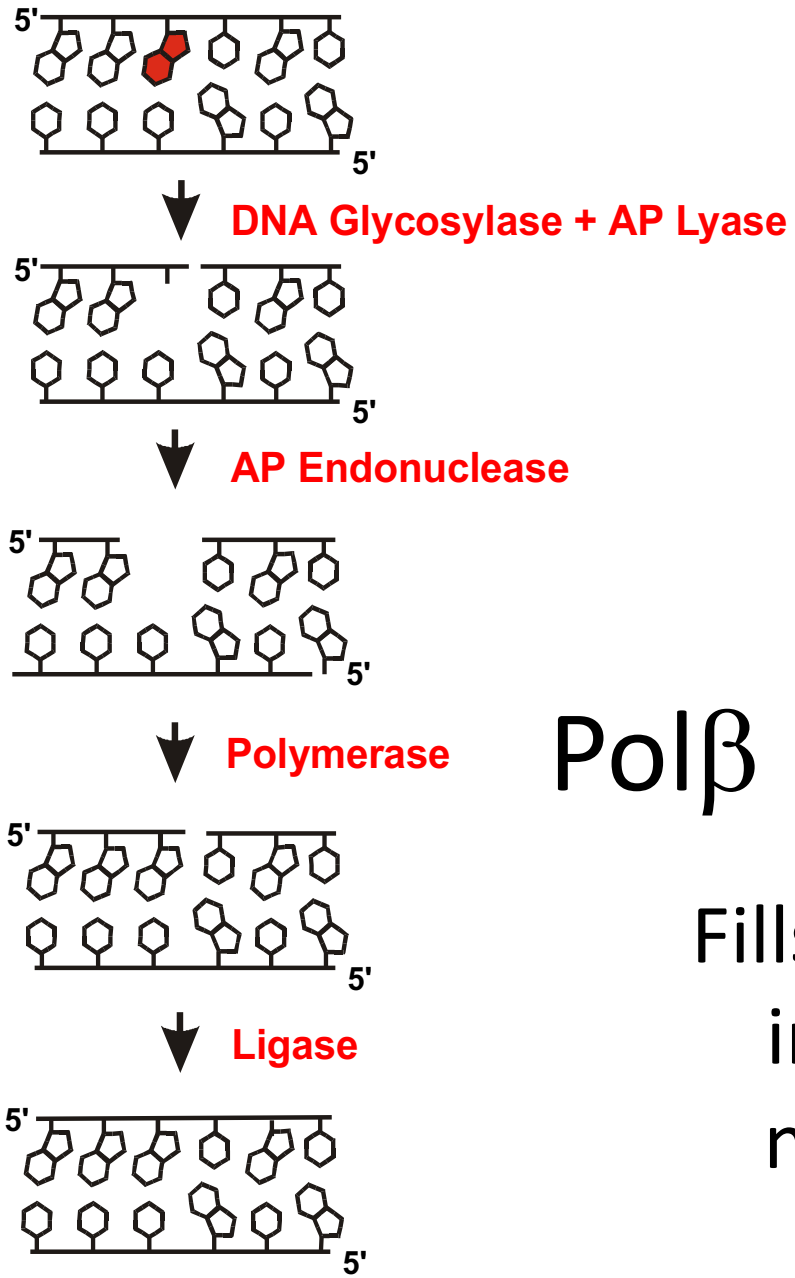
Creates a 3'OH that can be extended.



Single Nucleotide Addition by Polβ

Base Excision Repair

DNA Polymerase Beta

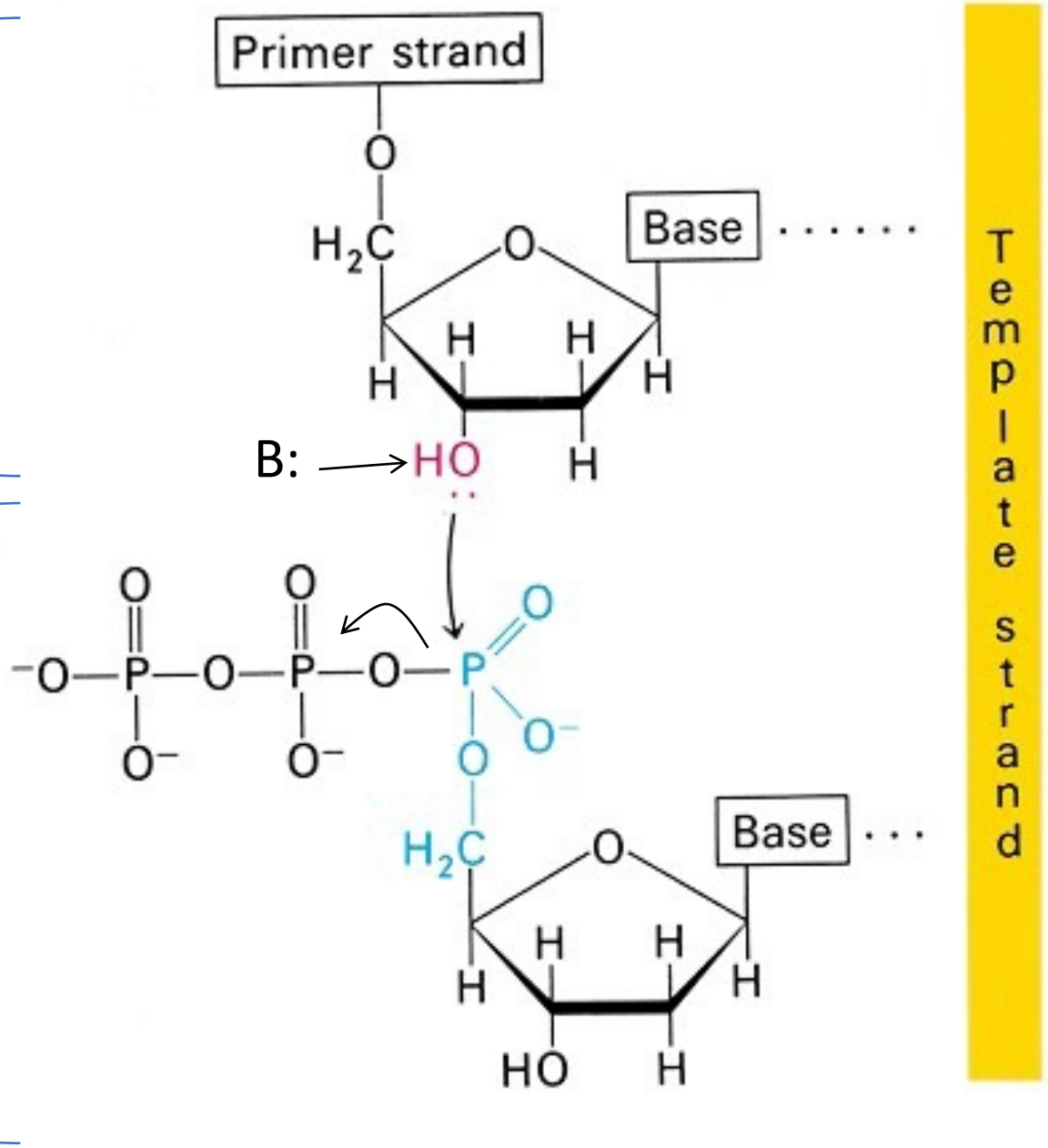


Polβ

Fills the gap by inserting a nucleotide

3’ End

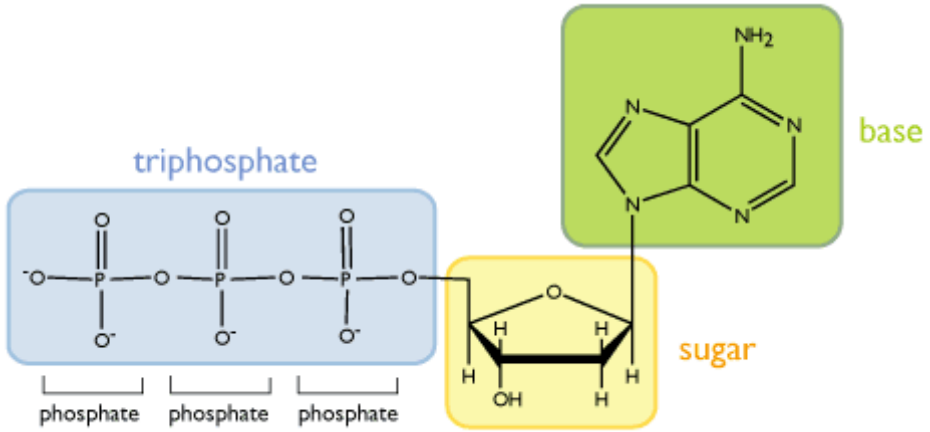
Incoming Nucleotide Triphosphate

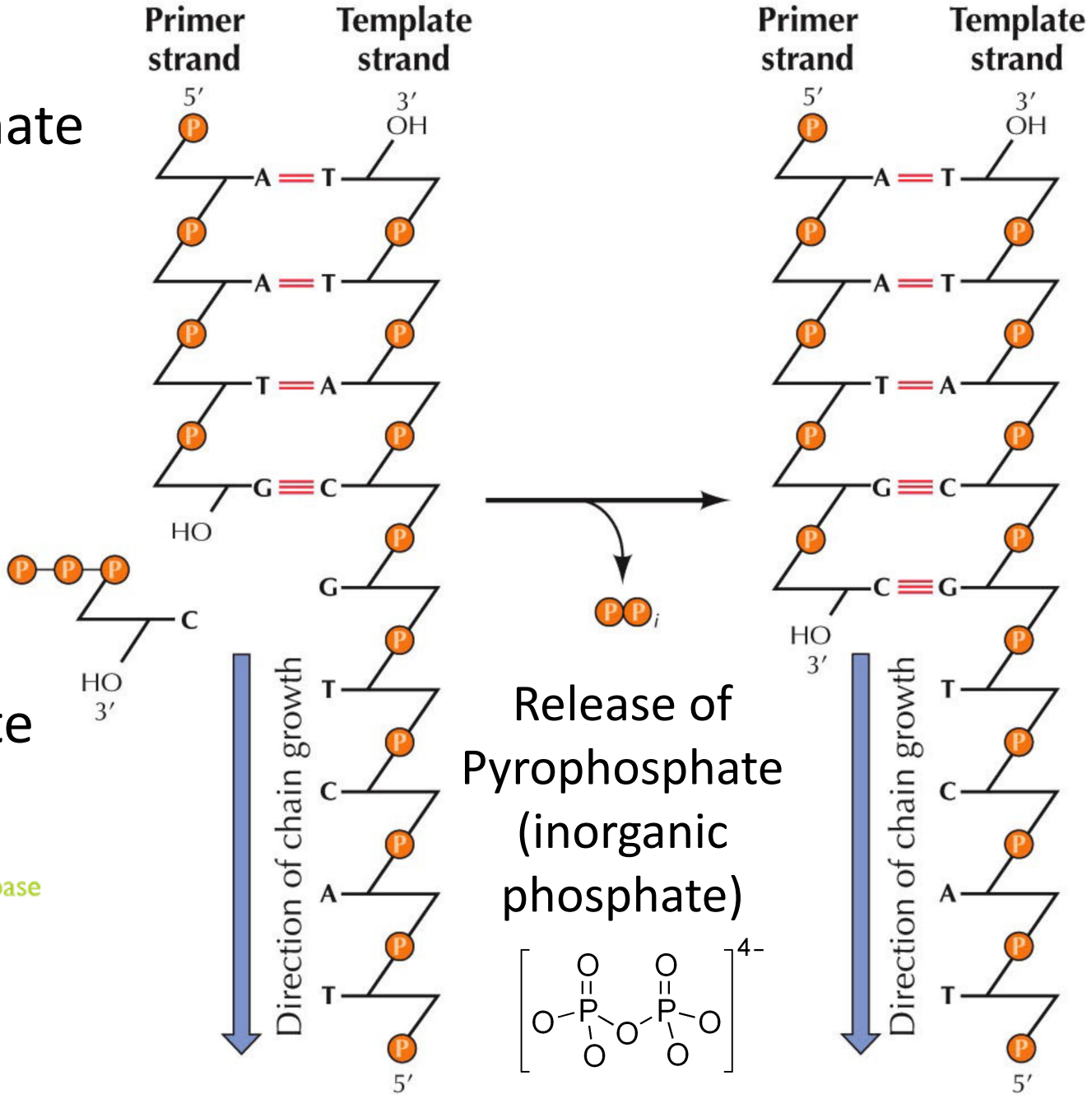


5’Phosphate

3’Hydroxyl

Nucleotide triphosphate





THE CELL, Fourth Edition, Figure 6.1 © 2006 ASM Press and Sinauer Associates

DNA Polymerase Beta (Polβ)

Mutations in Polβ in Mice cause Lupus-Like Symptoms – Possible association with Lupus in People but still *Unknown*

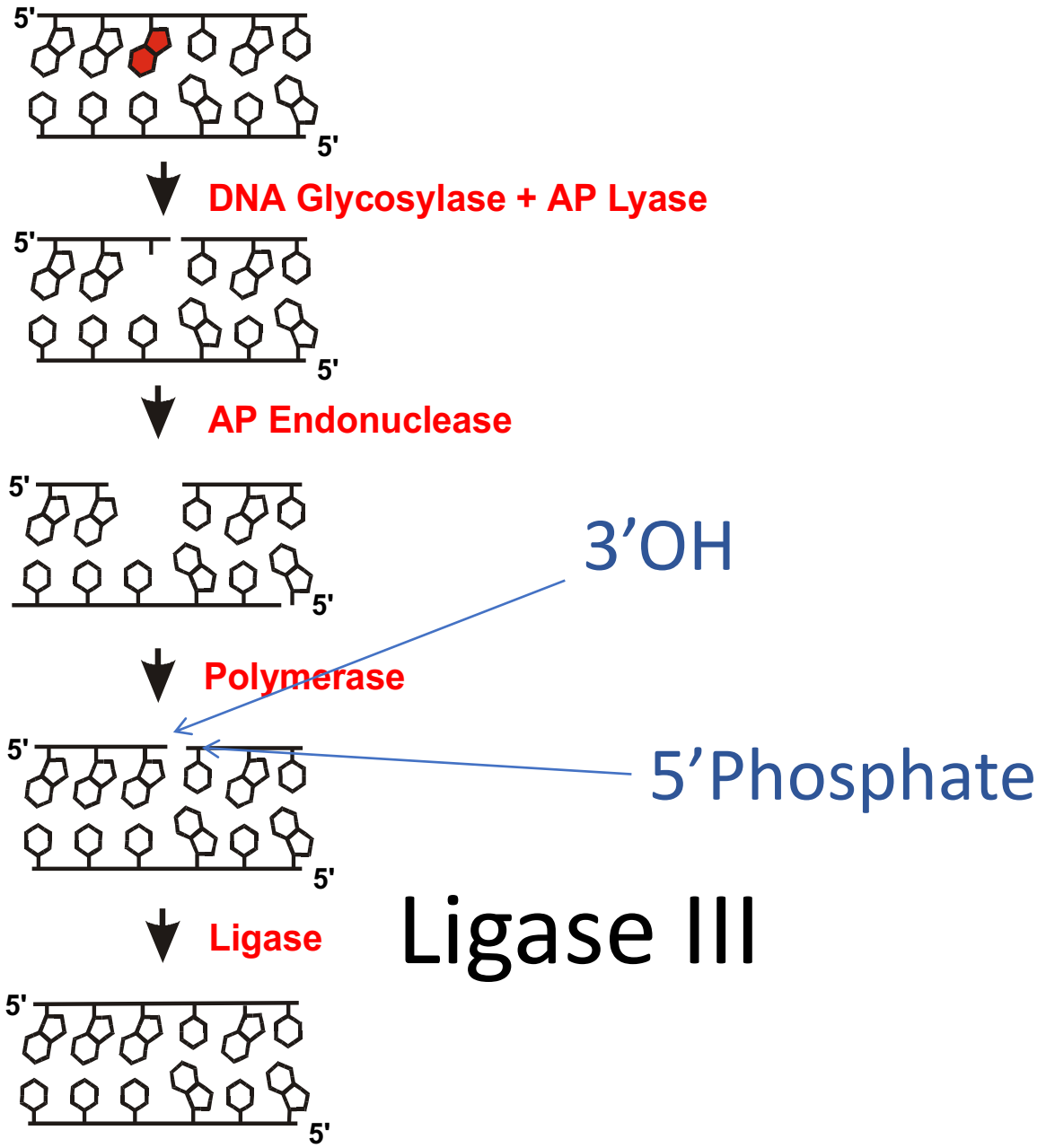
- Autoimmune disease
- Fatigue
- Fever
- Joint pain, stiffness and swelling
- Butterfly-shaped rash on the face
- Skin lesions that appear or worsen with sun exposure
- Fingers and toes that turn white or blue when exposed to cold or during stressful periods
- Shortness of breath
- Chest pain
- Dry eyes
- Headaches, confusion and memory loss

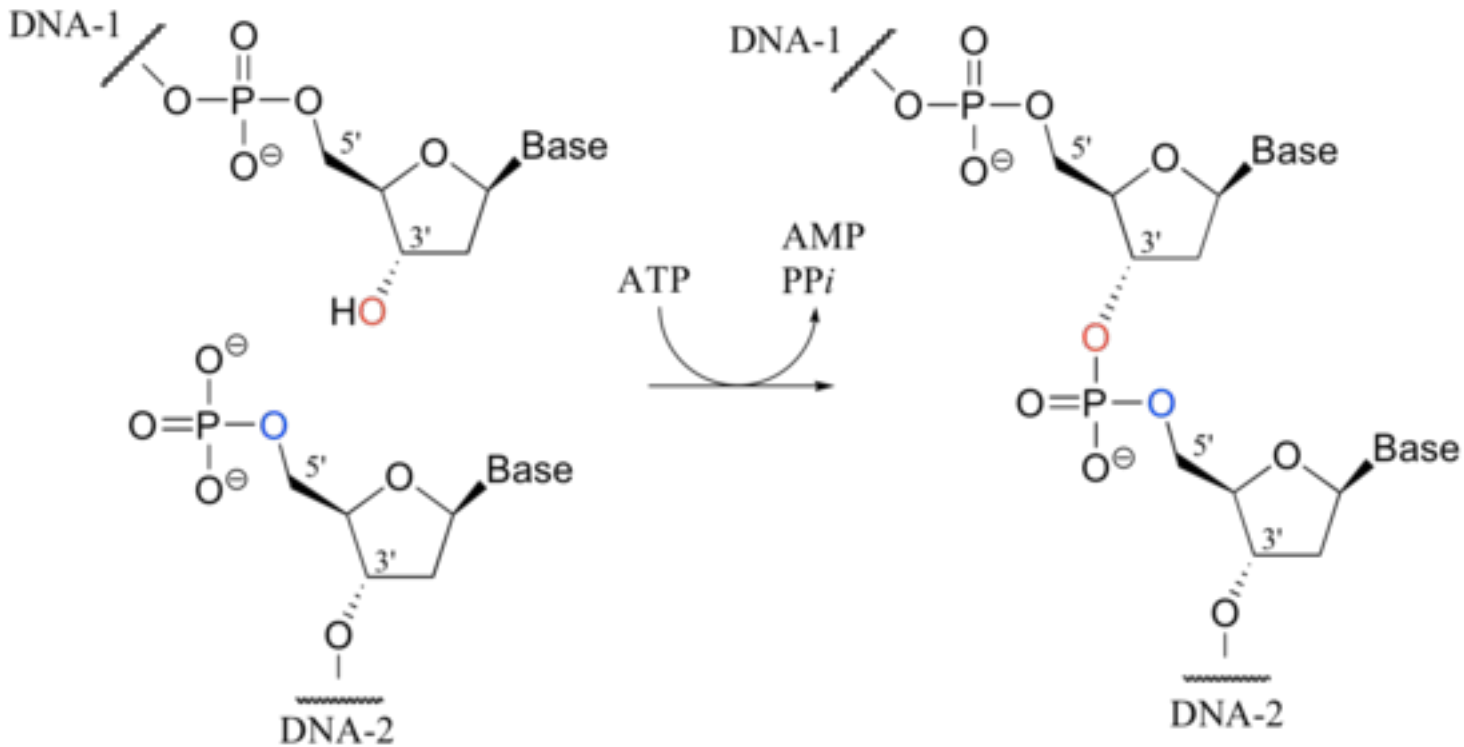
DNA Ligase III

Base Excision Repair

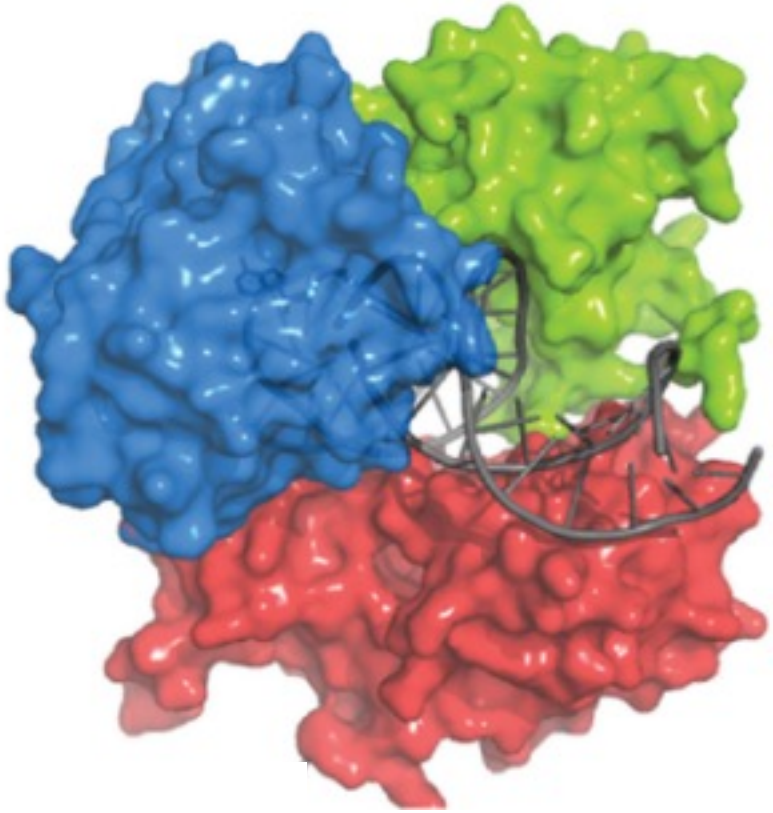
DNA Ligase III

Seals the nick by linking the 3’OH with the 5’Phosphate




DNA-1
O
O–P–O
O⊖
5'
O
Base
3'
HO
O⊖
O=P–O
O⊖
5'
O
Base
3'
O
DNA-2
ATP
AMP
PPi
DNA-1
O
O–P–O
O⊖
5'
O
Base
3'
O
O=P–O
O⊖
5'
O
Base
3'
O
DNA-2

Ligase III

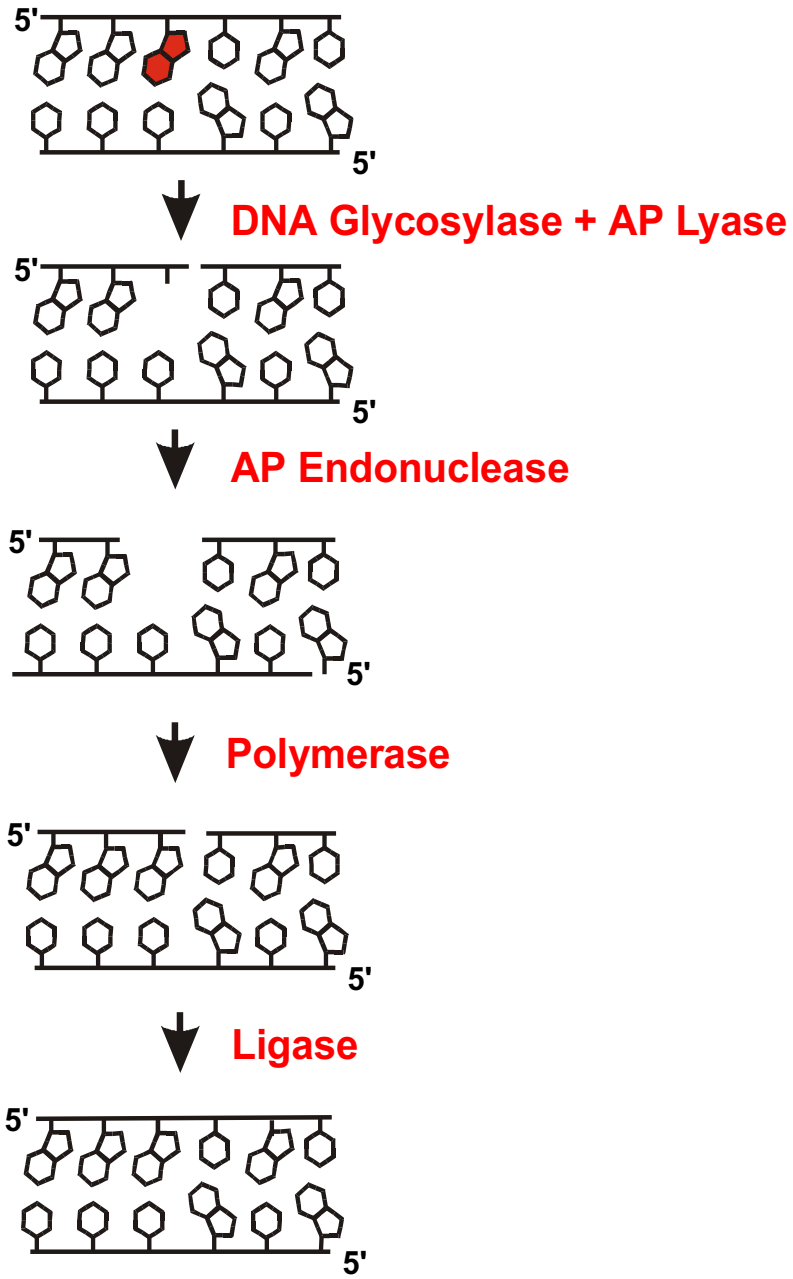


Pascali, O’Brien, Tomkinson, and Ellenberger, Nature 432: 473-478.

Mutations in Ligase III can cause Bloom's Syndrome

- Autosomal recessive
- Growth retardation
- Butterfly rash
- Defective immune system
- Increased risk of cancer

Base Excision Repair



Beautiful Pristine DNA!

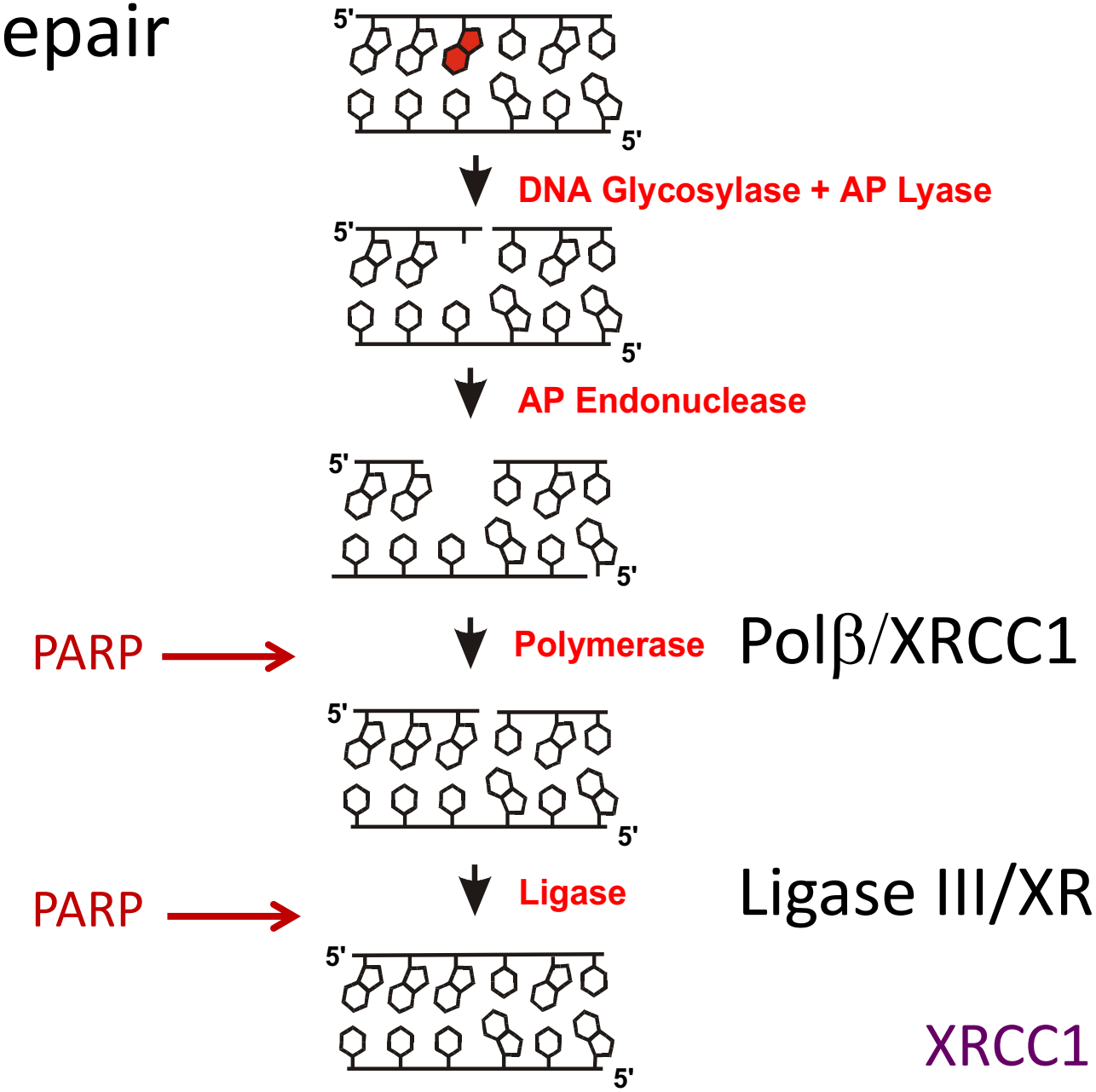
PARP

Poly(ADP-Ribose) Polymerase

PARP is a BER “Helper”

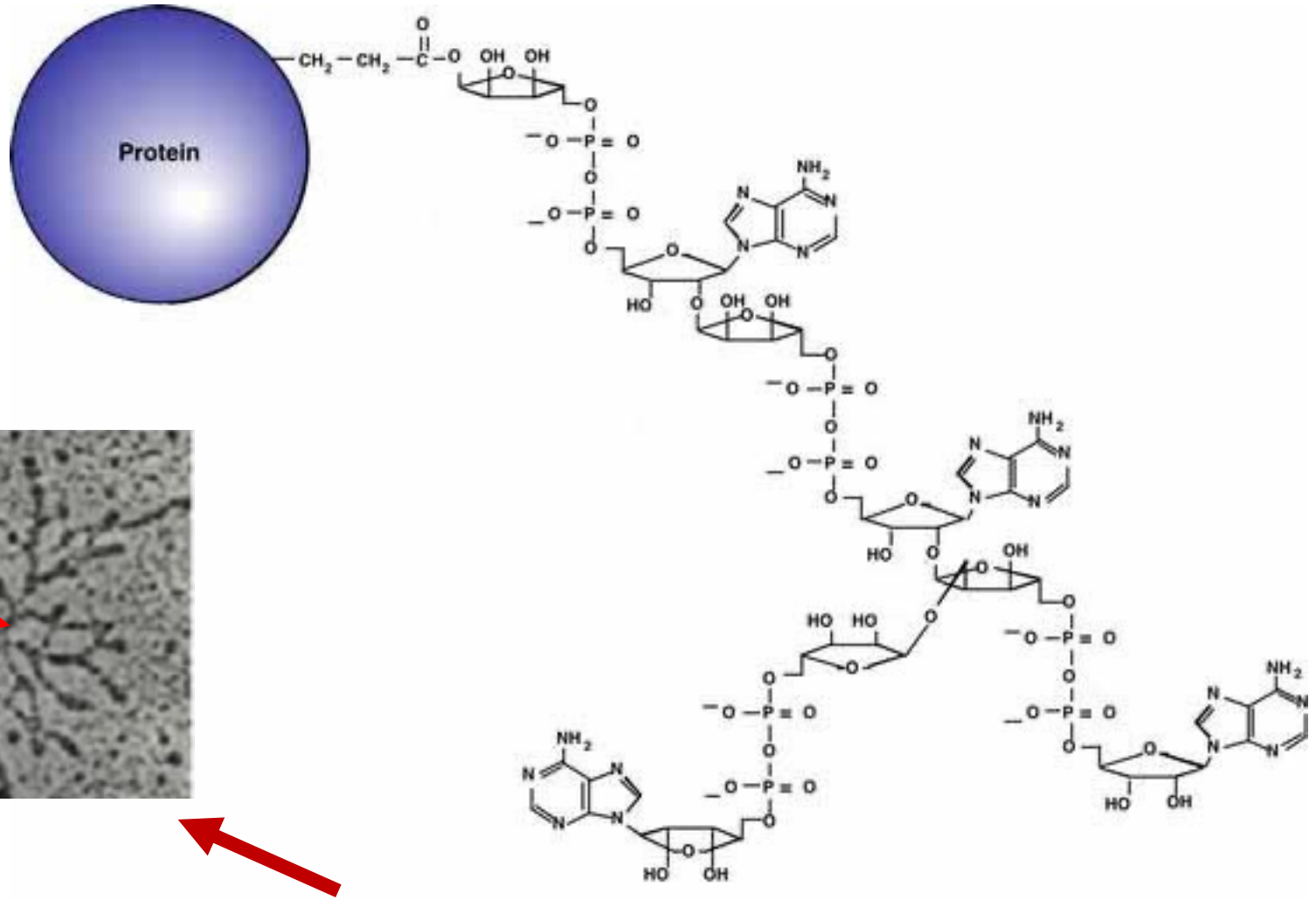
Accelerates BER

Base Excision Repair



XRCC1 = A Scaffold

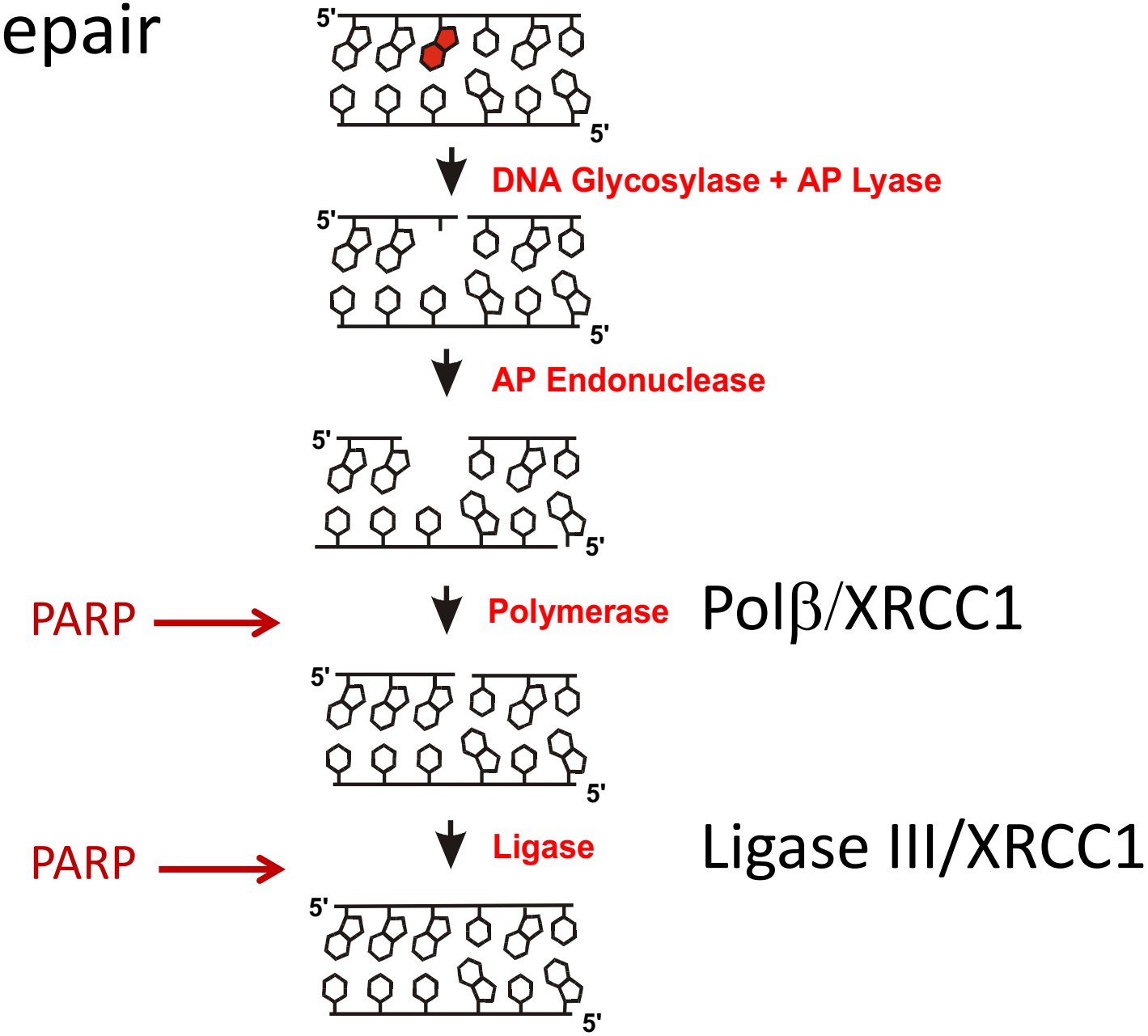
PARP Automodification Creates a Branched Structure



PARP is in the middle

Polβ/XRCC1 Ligase III/XRCC1

Base Excision Repair

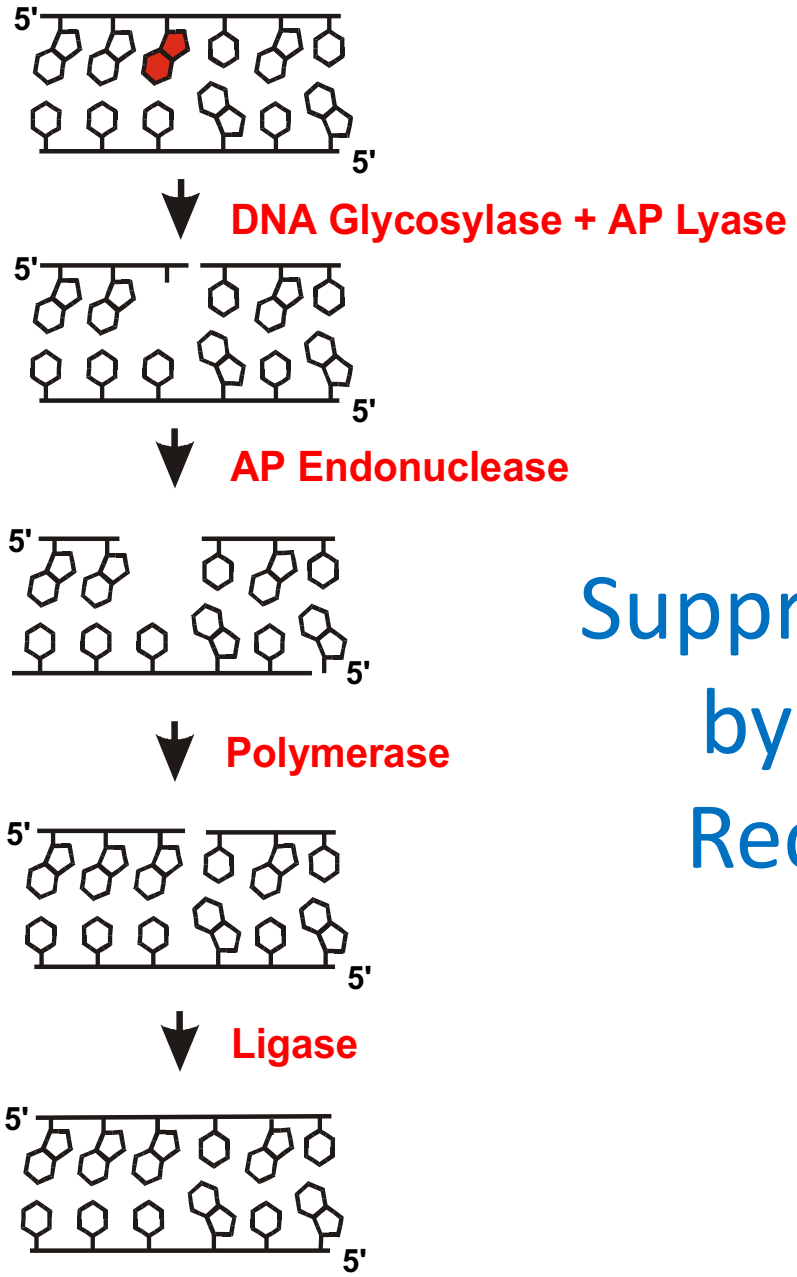


As Inhibits SSB Repair

As displaces zinc, disrupting zinc fingers in PARP

As ⊣ PARP →

As ⊣ PARP →



Suppression of PARP by As Reduces Recruitment of DNA Repair Proteins

As Inhibition of PARP leads to Increased Single Strand Breaks

Closely Opposed Single Strand Breaks lead to Double Strand Breaks

Summary

- Base excision repair requires multiple steps
- Key enzymes in BER are DNA glycosylase (OGG1), AP Endonuclease, Polymerase, and Ligase

- Polymerase requires a 3’OH
- Ligase requires a 3’OH and a 5’Phosphate

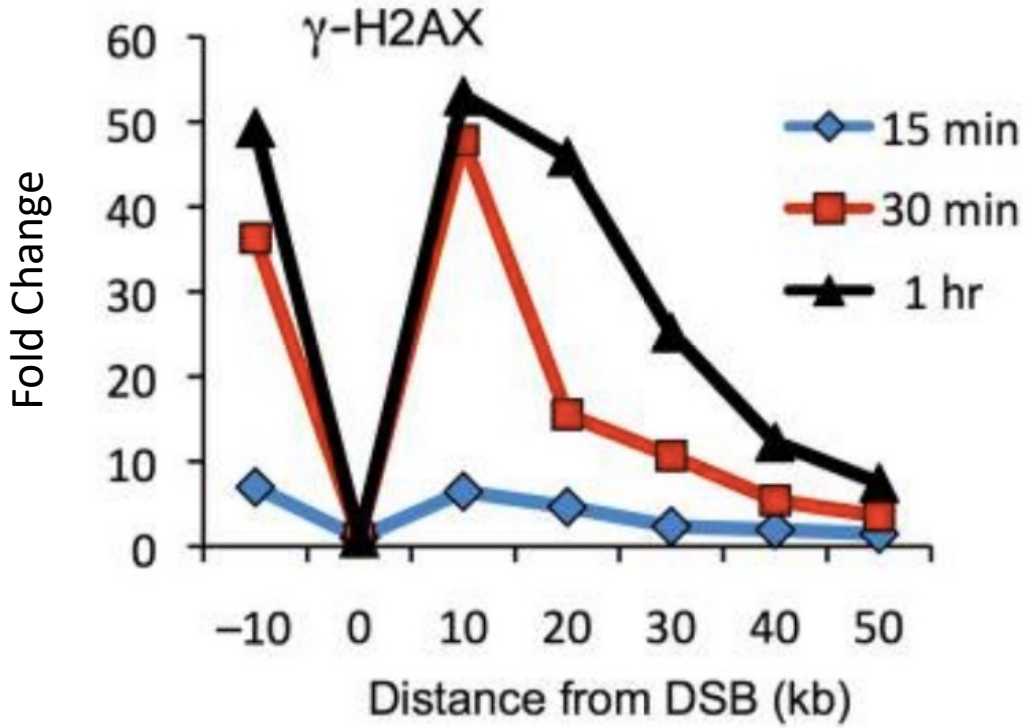
- PARP serves as a beacon to recruit BER enzymes
- PARP has a zinc finger and is inhibited when As replaces Zn
-

A careful look at the major steps of BER

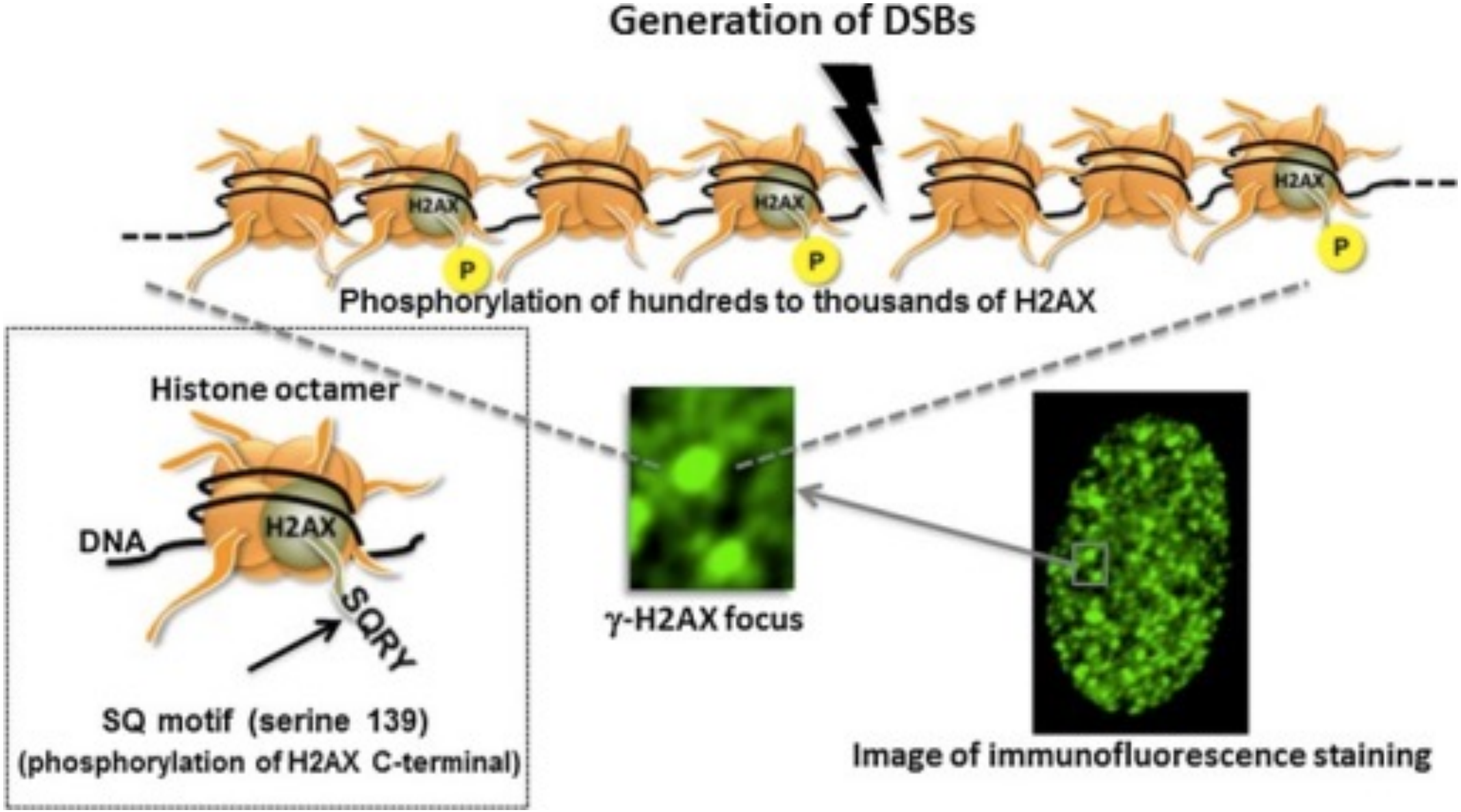
γH2AX as a Marker of DNA Damage

Interlude

Sensing DNA Damage with Antibodies



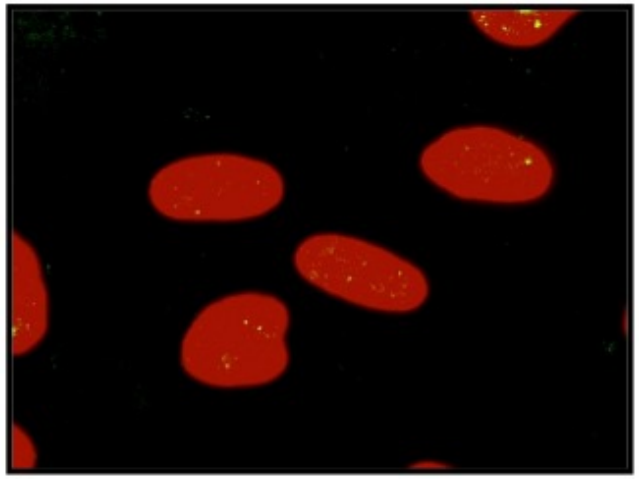
Study performed in *S. cerevisiae*
http://www.nature.com/nsmb/journal/v21/n1/fig_tab/nsmb.2737_F1.html



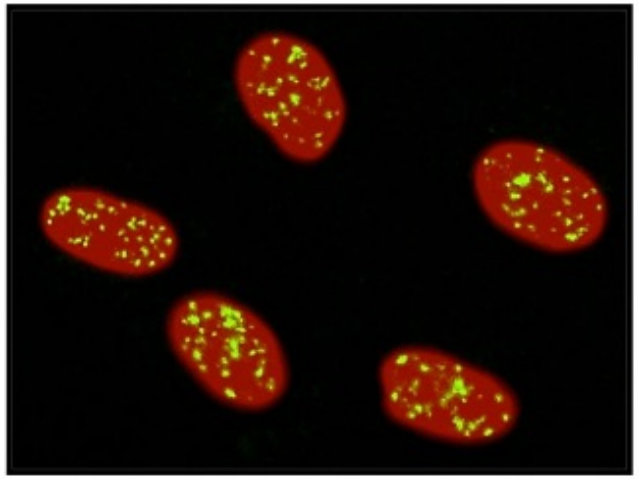
https://openi.nlm.nih.gov/detailedresult.php?img=PMC4380052_rru10502&req=4

γH2AX for Low versus High LET radiation

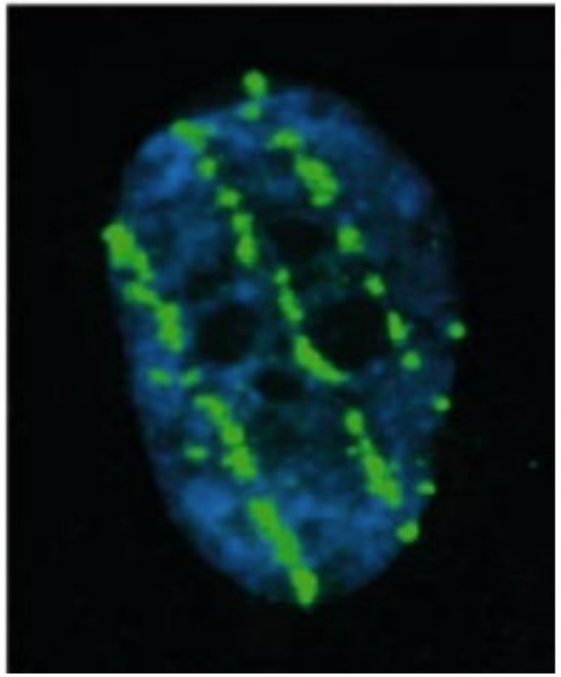
0 Gy



2 Gy



Low LET

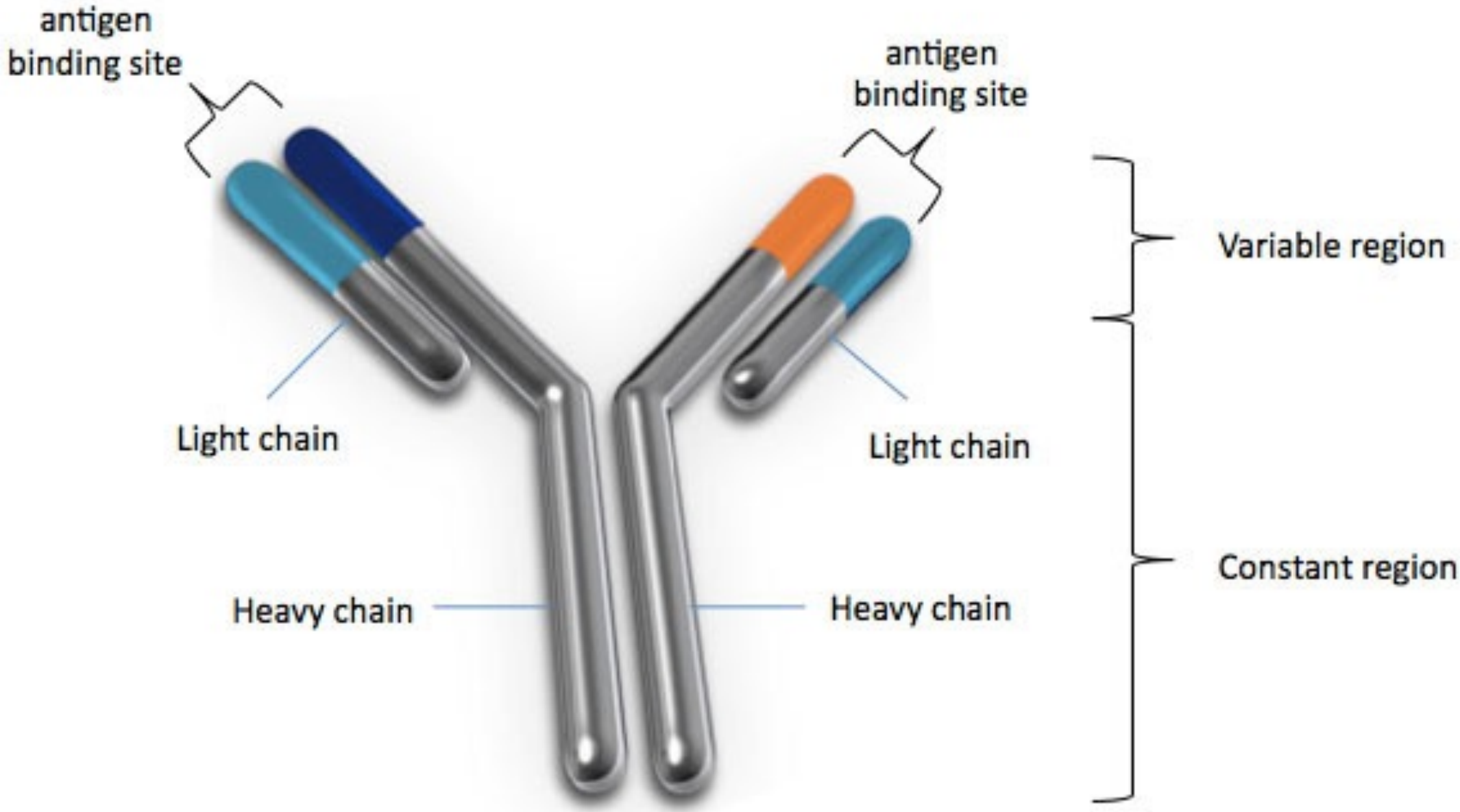


High LET

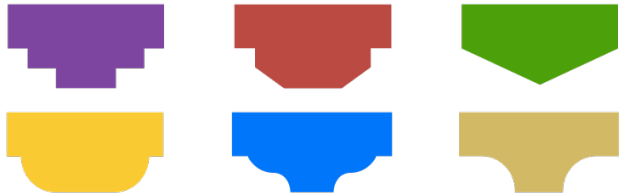
D Anderson et al 2013 JINST 8 C06008

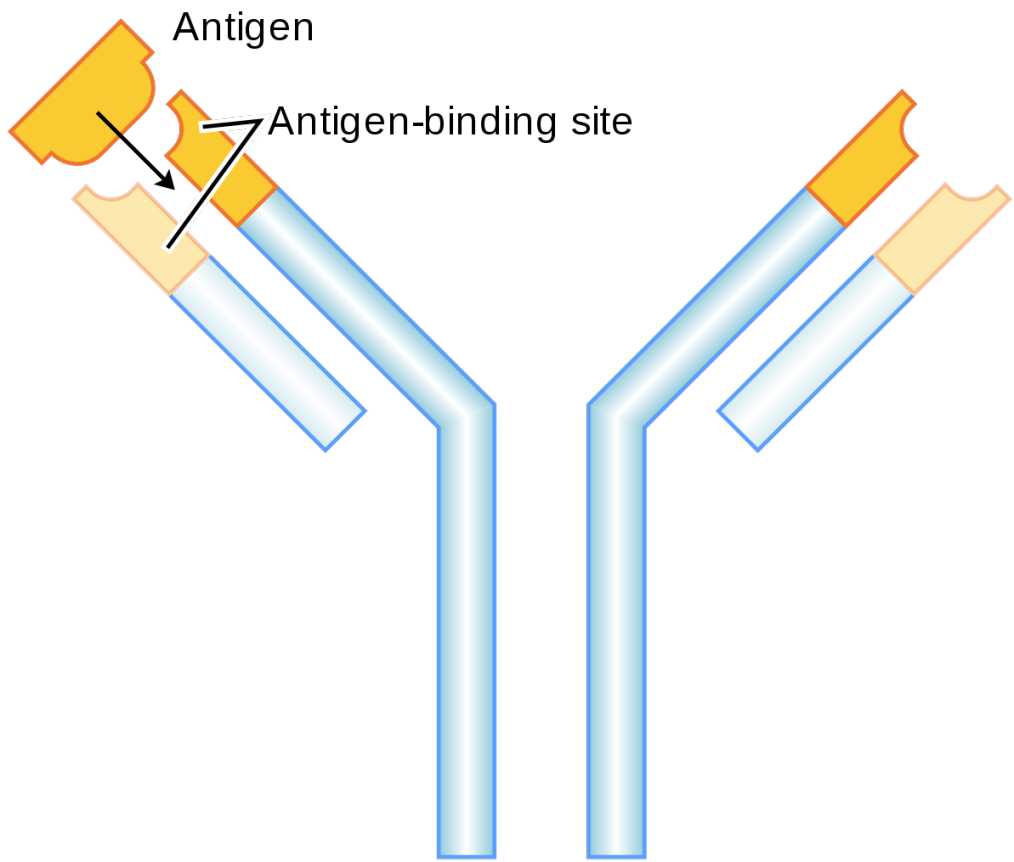
Desai et al., Rad. Res. 2005

Antibody Fundamentals

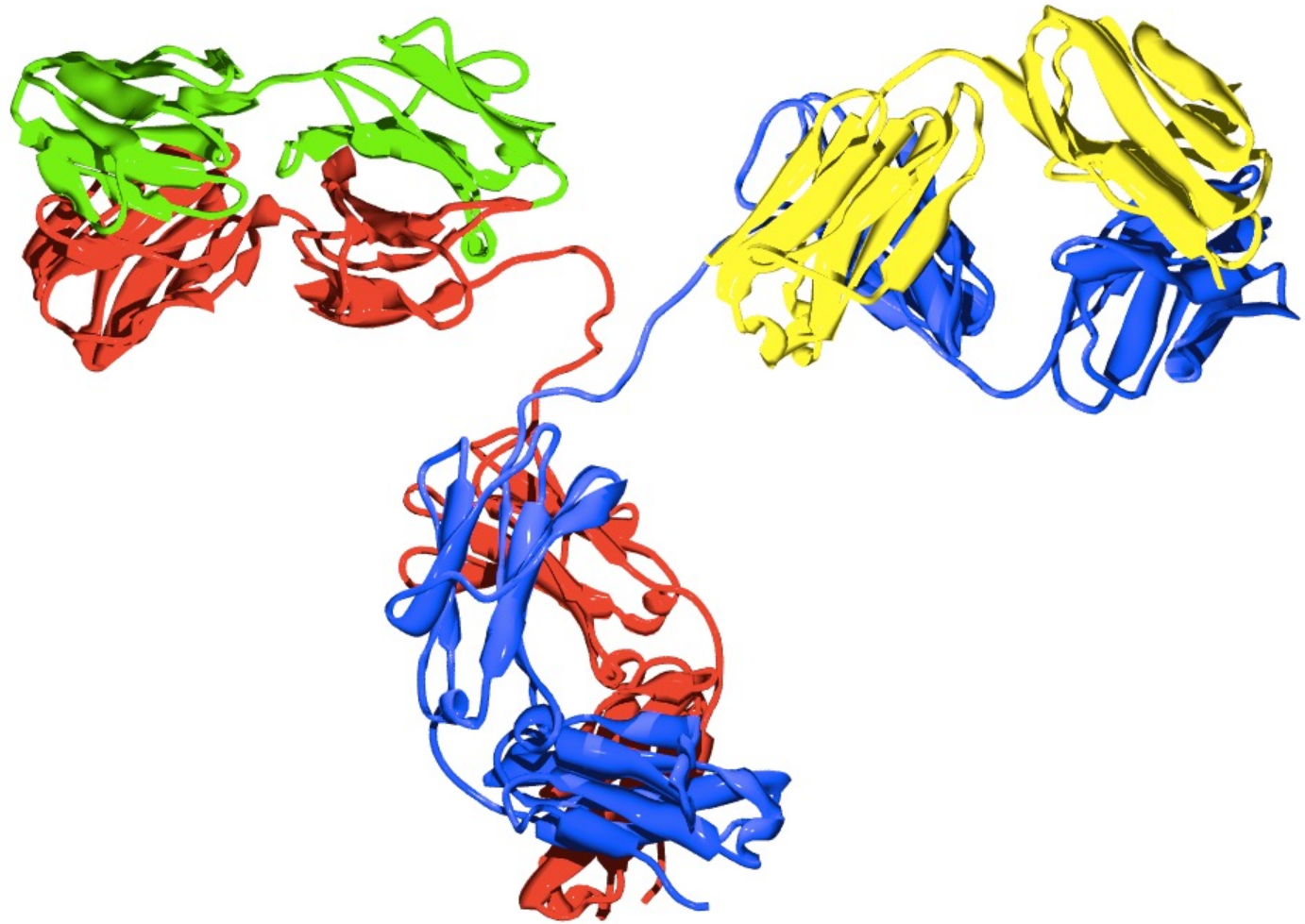

antigen
binding site
antigen
binding site
Variable region
Light chain
Light chain
Constant region
Heavy chain
Heavy chain

Antigens

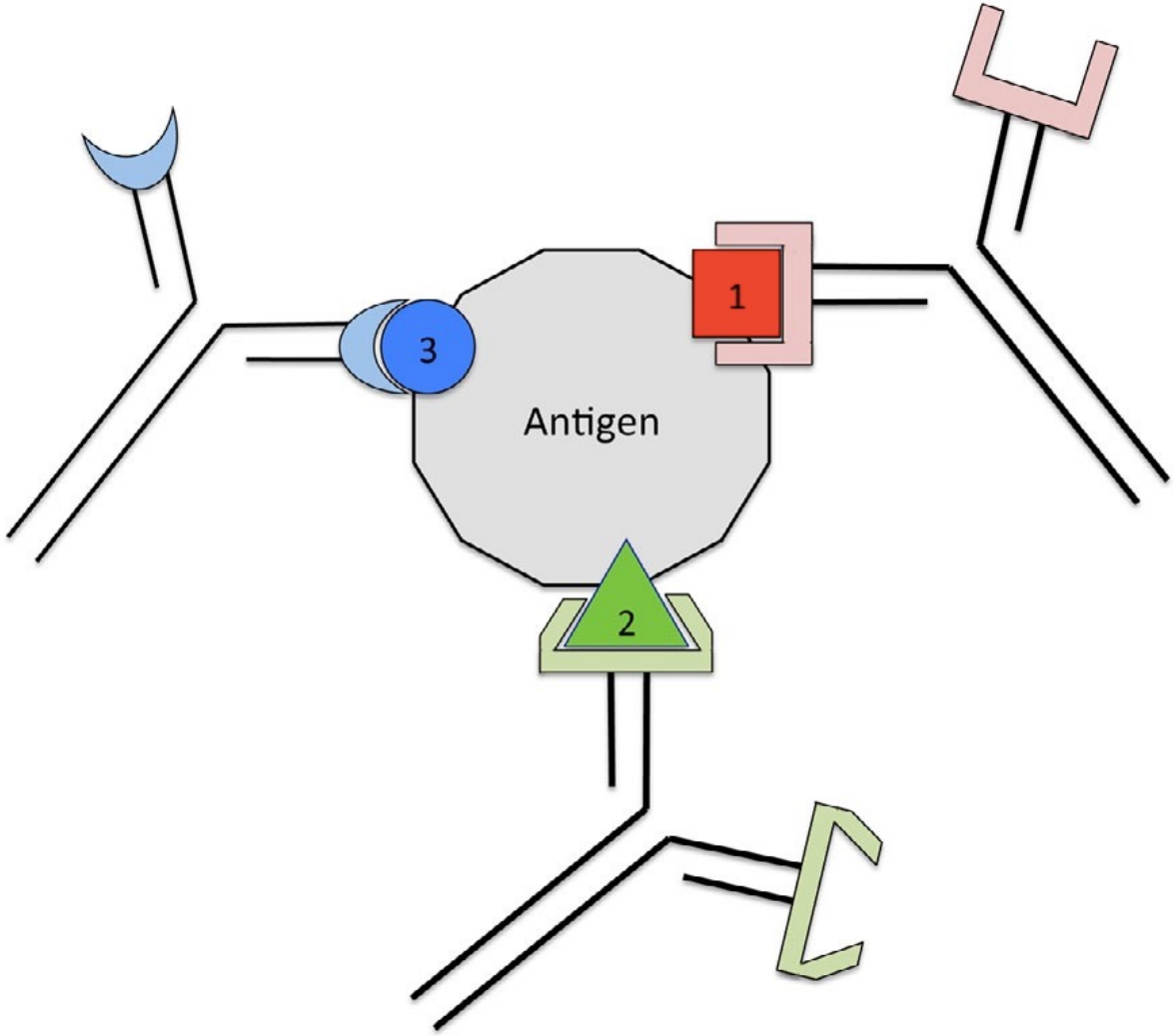




Antibody



Three Different Epitopes



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- PARP has a zinc finger and is inhibited when As replaces Zn

- H2AX gets phosphorylated when near DSBs to create γH2AX
- γH2AX serves as a beacon to recruit DNA repair enzymes